One Step Closer to Curing Tuberculosis: Testing the Stability of ORBIT in Mycobacterium smegmatis

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Tuberculosis is an infectious disease that kills 4,000 people a day worldwide. The bacteria that causes Tuberculosis, Mycobacterium tuberculosis, can easily mutate and develop drug resistance, making treatment difficult. Understanding the bacterial genome is essential for developing new treatments. Recombineering is a genetic engineering technique based on homologous recombination systems that is used to manipulate genomes. This experiment focuses on a recombineering technique called Oligonucleotide-Mediated Recombineering with Bxb1 Integrase Targeting (ORBIT). ORBIT is a site-specific genetic mutation technique that inserts an oligonucleotide sequence at the end of a target gene in the bacterial genome. The oligonucleotide contains an attachment site for a plasmid, harvested from a Bxb1 phage, that contains an antibiotic resistance marker. Discovered in 2015, ORBIT has the potential to be used to study the M. tuberculosis genome in order to develop more effective drug treatments. The exact stability of ORBIT in the bacterial genome is unknown. This experiment tested the stability of ORBIT in both nonessential and essential genes in Mycobacterium smegmatis, a non-pathogenic bacteria often used for the study of M. tuberculosis. Bacteria containing the Bxb1 plasmid (pKM468-eGFP) was plated over generations, on both plain 7H10 agar, and 7H10 agar containing hygromycin, the antibiotic for which the plasmid contains a resistance marker. At the end of experimentation, the investigator performed PCR and microscopic analysis to confirm the correct positioning of the plasmid. In conclusion, the use of ORBIT at the end of essential and nonessential genes is extremely stable in M. smegmatis. Therefore, ORBIT holds excellent promise for tuberculosis research.

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